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DINITROPHENOL AND BIOENERGETICS: AN HISTORICAL PERSPECTIVE

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Summary

The subject of the paper is the history of dinitrophenol compounds in relationship to bioenergetics. The history of the interaction between dinitrophenols and bioenergetics can be traced back to 1885 when CAZENEUVE and LÉPINE discovered the thermogenic effects of dinitronapthol. Dinitronapthol and dinitrocresol were used as food colors in the late 19th century although a growing awareness of their toxic properties led to the prohibition of their use for this purpose in certain countries. The toxicity of dinitrophenol was studied in some detail by MAYER and his colleagues in France during World War I since it was used by the French in the manufacture of munitions. They recognized that the compound stimulated cellular metabolism, but they did not publish their results until many years later and as a result their work was at first not widely known. In the late 1920's and early 1930's, Corneille HEYMANS and his colleagues at Ghent and CUTTING and TAINTER and their colleagues at Stanford demonstrated the metabolic stimulating powers of dinitronapthol and dinitrophenol. The Stanford group introduced dinitrophenol into therapeutics for the treatment of obesity, and the drug soon found its way into numerous "anti-fat" patent medicines. Several fatalities, a number of cases of cataract, and other reported toxic effects led to widespread concern about the use of dinitrophenol. The FDA could not take effective action against the drug, however, until after its powers had been expanded by the 1938 Food, Drug and Cosmetic Act. The use of dinitrophenol and related compounds for treating obesity was essentially discontinued after the 1930's. Studies on the mode of action of dinitrophenol in the 1930's and 1940's led ultimately to the establishment of the fact that it uncouples oxidative phosphorylation (LOOMIS and LIPMANN, 1948).

Introduction

Probably the best-known agent for uncoupling oxidative phosphorylation is 2,4-dinitrophenol. Because of its mode of action, the history of the pharmacology and toxicology of this compound is closely connected to the history of bioenergetics. The purpose of this paper is to examine the history of dinitrophenol, and related compounds (specifically dinitronapthol and dinitrocresol), in relationship to bioenergetics.

Early Work on the Toxicity of Nitrophenols

The history of the interaction of dinitrophenol compounds and bioenergetics can be traced back to 1885 when two French scientists, P. CAZENEUVE and R. LÉPINE, first reported the thermogenic effect of dinitro-alpha-napthol[1]. CAZENEUVE and LÉPINE were interested in the physiological action of dyes which were used in coloring foods and beverages. One of the dyes which they studied was Martius YELLOW, or dinitronapthol, a coaltar dye which was used in the late nineteenth century to color products such as macaroni and pastries (the yellow color created the appearance that the product was rich in eggs). It was also used to dye wool and silk. The sodium salt of dinitronapthol (the

form in which the dye was commonly employed) was administered to dogs, and the symptoms observed, if the dose were sufficient, were vomiting, diarrhea, increased respiration, a marked rise in temperature, and finally death. The temperature rose in one case as high as 44 °C. CAZENEUVE and LÉPINE noted that the respiration was increased without any decrease in the normal proportion of oxygen in the blood, and that the temperature was increased without convulsions. This latter observation suggests that they were aware that the rise in temperature was not due to muscular activity. They also noted that dinitronapthol-monosulfonic acid, which was also used as a dye under the name of Yellow S or Naphthol Yellow S was not appreciably toxic. It is not an uncommon phenomenon for the toxicity of a substance to be destroyed or greatly reduced by the addition of a sulfonic acid group.

Another dinitrophenol derivative of interest to us, dinitrocresol, also found use as a dye in the nineteenth century. Under the name of Victoria Yellow (or Orange) or Saffron Substitute, it was used for much the same purposes as Martius Yellow. The toxic properties of this substance was apparently first recognized by Theodore WEYL, who studied its action in rabbits and dogs[2]. WEYL also reported on a case he had investigated in which a woman had died after the ingestion of Victoria Yellow in the belief that it was saffron. It is curious, however, that WEYL did not report a rise in temperature as one of the effects of dinitrocresol. He does not record the temperatures of the experimental animals who received the drug, and apparently made no attempt to measure body temperature. Yet he also tested the pharmacological effects of dinitronapthol and here he confirmed the observations of CAZENEUVE and LÉPINE on the thermogenic effects of this dye. Probably his awareness of the earlier work of his French colleagues alerted him to specifically look for a temperature rise in the case of dinitronapthol, but it is still curious that he did not test dinitrocresol for this property since it was known to be structurally similar to dinitronapthol.

Wolcott GIBBS and Edward REICHERT experimented with dinitrophenol on frogs and dogs in the 1890's, and reported that it was quite toxic[3, 4]. While they did mention that it caused a rise in temperature and a stimulation of respiration, as well as a rapid onset of rigor mortis upon death, these observations received only passing notice in their account. They emphasized instead other properties of the compound, reporting that it caused death by heart failure when administered by the jugular vein and by respiratory paralysis when given by mouth. The drug was given in relatively large quantities (up to 0.17 g per kg of body weight intravenously and up to 0.3 g per kg orally) and this factor probably caused GIBBS and REICHERT to overemphasize respiratory and cardiac inhibition. Large doses of dinitrophenol have been observed to inhibit respiration, and massive doses given intravenously have been reported to cause cardiac failure[5, 6]. Because it was not used to any significant extent as a dye, although it was used as an intermediate in dye production, the toxicity of dinitrophenol did not receive as much attention as the toxicity of the nitrophenols previously mentioned.

After the recognition of their toxic properties, the use of dinitronapthol and dinitrocresol as dyes for foodstuffs was prohibited by law in a number of European countries in the late nineteenth century[7, 8]. In the United States, Martius Yellow and Victoria Yellow were not included in the seven coal-tar dyes which were permitted to be used as food colors under Food Inspection Decision No. 76, 1907[9]. When Martius Yellow was detected in a shipment of macaroni seized by food inspectors for false labelling, Harvey WILEY, head of the USDA Bureau of Chemistry, asked A. P. MATHEWS, a physiological chemist at the University of Chicago, to test the toxicity of the dye. In 1910, MATHEWS and a coworker, Elizabeth LONGFELLOW, published a paper on Martius Yellow in which they reviewed the earlier literature on this substance and reported the results of their own experiments, which confirmed its toxicity, including its effect on body temperature and respiration[10]. The early onset of rigor mortis upon death was also noted. The authors also cited two cases of poisoning from ingestion of dinitronapthol which had been reported in the literature.

This incident was not to be the last time that a dinitrophenol compound attracted the attention of those responsible for the enforcement of food and drug laws in the United States.

Dinitrophenol and Munitions Manufacture

Interest in the toxicity of dinitrophenol was stimulated during World War I as a result of its use in the

manufacture of munitions. Trinitrotoluene was not used as extensively as an explosive in France as in Britain and the United States. The French more commonly used a mixture of 40% dinitrophenol and 60% trinitrophenol (picric acid) as an explosive. After the appearance of a number of cases of intoxication in French munitions works, the Ministry of Munitions organized in 1915 a Commission for the Study of the Toxicity of Explosives. The Commission reviewed the literature on the toxicity of various explosives and made clinical studies of workers suffering from intoxication, and in addition undertook pharmacological experiments on explosives under the direction of Dr. André MAYER, assistant director of L'École des Hautes Études. The Commission's report was delivered in June, 1918, but was not published. The essential results of their studies on dinitrophenol were reported, however, in 1919 in an article in *Public Health Reports* by Rogers PERKINS, Medical Associate to the Scientific Attaché to the American Embassy in Paris, and in an M.D. thesis presented to the Faculty of Medicine of the University of Paris by Edmond QUIGNARD[11, 12].

Both pharmacological experiments on animals and clinical observations on humans indicated that dinitrophenol was a highly toxic agent. The symptoms of intoxication included a stimulation of respiration and a rise in body temperature, as well as the almost immediate onset of rigor mortis upon death. MAYER and his colleagues indicated that the rise in temperature was due to an increase in heat production rather than to a decrease in the radiation of heat. In fact, the heat radiation activities of the body were actually increased during dinitrophenol intoxication, e.g., vasodilation and profuse sweating occurred. Dinitrophenol produced no increase in muscular work, so muscular activity was apparently not the source of increased heat production. Nor was the rise in body temperature due to a stimulation of the thermoregulator system; it occurred even in animals which were deeply anesthetized or whose spinal cord had been severed, as well as in cold-blooded animals. The increased heat production, they concluded, must be due to a stimulation of cellular oxidation, the quantity of oxygen consumed being increased five- and even tenfold. They also observed a disappearance of the glycogen reserves of the body.

With the end of the war, dinitrophenol became less important and of less concern, although the work of MAYER and his colleagues had led to some recognition of the need for safety precautions in the handling of dinitrophenol[13, 14].

Interest in the Nitrophenols as Metabolic Stimulants

The study of the pharmacology and toxicology of dinitrophenol compounds was renewed in the late 1920's by Corneille HEYMANS and his coworkers at the Laboratory of Pharmacodynamics and Therapy of the University of Ghent. HEYMANS' interest in agents which stimulate metabolism goes back to 1922, when he noted that methylene blue produced an augmentation of cellular metabolism and a rise in body temperature. Beginning in 1928 and continuing on into the early 1930's, he and his collaborators, aware of the work of CAZENEUVE and LÉPINE, WEYL, and MATHEWS and LONGFELLOW, turned their attention to the metabolic stimulating properties of dinitronaphthol. Later, dinitrophenol and dinitrocresol were also investigated. Numerous preliminary reports were published in the *Comptes rendus* of the Société de Biologie from 1928 on, and much of this work was summarized in a 1935 article[15].

In their work on dinitronapthol, HEYMANS' group confirmed earlier observations on the toxicity of this substance but greatly elaborated on the knowledge of its action. They clearly showed that the fever produced by dinitronapthol was due to a direct stimulation of cellular metabolism. The administration of this substance was not accompanied by an increase in muscular activity or in the retention of heat (in fact, heat radiation was increased), and a rise in temperature occurred even when the animal had been deprived of its thermoregulation center. They concluded that the stimulation of heat production by dinitronapthol was of a peripheral rather than of a central origin, i.e. it was caused by a direct stimulation of cellular metabolism rather than by a stimulation of the central nervous system. The Ghent workers were apparently unaware of the work of MAYER and his colleagues, which had never been published by the authors although it had been reported to some extent in the literature, which had postulated a similar mechanism of action for dinitrophenol.

HEYMANS and his colleagues also performed various biochemical studies which indicated that the increase in temperature caused by dinitronapthol was accompanied by a diminution of muscular and hepatic

glycogen, an augmentation of the phosphate content of blood and muscle, an increase in the lactic acid content of muscle, and a decrease in the phosphocreatine content of muscle. While these findings are significant in light of our modern understanding of biological phosphorylation and of the mechanism of action of dinitronapthol, they could not at the time lead to an explanation of the increased heat production caused by this agent. When HEYMANS and his colleagues began their work in 1928, of course, the discovery of ATP was still a year away and an understanding of oxidative phosphorylation was still a number of years in the future.

By 1931, HEYMANS had apparently investigated the action of dinitrophenol and demonstrated that it also produced fever in experimental animals, for he informed two Stanford University scientists of this fact in a personal communication[16]. The two scientists, W. C. CUTTING of the Department of Medicine and M. L. TAINTER of the Department of Pharmacology of the Stanford University School of Medicine, decided to study the cause of the temperature rise and its possible therapeutic applications. Over the next several years, CUTTING and TAINTER and their colleagues subjected dinitrophenol to extensive pharmacological and clinical testing.

Unaware at first of the earlier investigations of MAYER and his coworkers, they repeated their work and also found that dinitrophenol produces a rise in temperature and an increase in respiration. Since the rise in temperature was not prevented by the destruction of the brain and the spinal cord or by complete curarization, they concluded that the mechanism of production was a peripheral one[17, 18].

While CUTTING and TAINTER were performing their pharmacological studies, André MAYER and his colleagues finally published the results of their work, performed some fifteen year earlier on dinitrophenol[19, 20]. Both teams of investigators had arrived at essentially the same results independently. The Stanford group continued, however, to study in more detail the pharmacology and toxicology of dinitrophenol, and especially its possible therapeutic uses.

In 1933, they reported on their first clinical tests, which indicated that in daily oral doses of 3 to 5 mg per kg of body weight the drug maintained the metabolic rate of patients at an average of 40% above the initial level[21, 22]. All of the subjects consequently lost body weight without resort to dietary restrictions. The patients did not seem to suffer any deleterious effects from this treatment. The Stanford workers thus suggested that the drug might be useful in the treatment of obesity, hypothyroidism and similar depressed metabolic states. In light of its toxic properties, however, they warned that for the time being it should be used only as an experimental therapeutic procedure in carefully selected patients under close observation by the physician.

But the promise of a drug which could reduce body weight without any dietary restriction proved to be too tempting to a figure-conscious public, to physicians pressured for miracle cures by obese patients, and to profit-conscious patent medicine promoters, and the warning of the Stanford team went unheeded.

A little more than a year after their first clinical report on dinitrophenol had appeared, the Stanford team expressed their concern over the unrestricted use of the drug before the Food and Nutrition Section of the American Public Health Association[17]. They estimated that probably at least 100,000 persons had been treated with the drug in this country, as well as many others abroad. Upwards of 20 wholesale drug firms were marketing the compound, and it had found its way into various self-medication products.

Dinitro-o-cresol was also being used for the treatment of obesity after being introduced for this purpose by E. C. DODDS, W. J. POPE, and J. D. ROBERTSON in England in 1933[23, 24]. A disagreement developed between the English workers and the Stanford group as to which of the two compounds, dinitrophenol or dinitrocresol, was less toxic and more effective[23, 24, 25]. HEYMANS' group also entered the fray and supported the use of dinitrocresol or preferably dinitrothymol, because their studies indicated a lower toxicity of these compounds (especially the latter) relative to dinitrophenol and dinitronapthol[26].

Public Concern over Dinitrophenol in the United States

In the United States, public concern over dinitrophenol was stimulated by the reports of several deaths attributed to dinitrophenol. "Diet and Die with Excess Alpha Dinitrophenol" warned *Newsweek* in 1933, as they recited the story of a physician who had been "literally cooked to death" when he took an overdose of the drug in an attempt to lose weight rapidly[27].

In 1935, another potential danger of dinitrophenol use was recognized when several cases were reported of women developing cataracts while receiving dinitrophenol for weight reducing purposes[28, 29]. Other toxic effects, such as agranulocytosis were also noted[5]. A number of medical researchers also began to question the actual effectiveness of dinitrophenol in treating obesity as compared to even moderate dietary restriction[30, 31]. The *Journal of the American Medical Association* warned readers of the potential dangers of the drug and recommended that its use be restricted to controlled studies in hospitals with physicians competent to evaluate the drug and laboratory facilities available for determining changes in the body tissues[32]. One problem in its widespread use according to one observer, was that physicians, and certainly patients, were not generally in a position to monitor the basal metabolism rate to insure that metabolism was not being increased too drastically[33]. It was not safe to assume that the production of a fever would serve as an adequate warning to lower the dosage since the ratio of a fatal dose to a pyretic dose was not very high. For example, CUTTING, TAINTER and BERGSTROM found that in rats the ratio of the dose killing 50 per cent of the animals to the dose causing a fever of 2 °C was only 1.7[34].

The introduction of dinitrophenol into self-medication products was of particular concern since these drugs could be taken by the patient with no medical consultation. The presence of dinitrophenol was also often not revealed on the labels of the "anti-fat nostrums." The Bureau of Investigation of the American Medical Association and the Food and Drug Administration discovered at least twenty patent medicines which contained dinitrophenol or a related compound such as dinitrocresol[35, 36, 37].

The 1930's was the era of the "guinea-pig muckrakers", authors of books aimed at exposing the exploitation of the consumer in the American market[38]. Drugs and cosmetics came in for their share of criticism by these authors, and dinitrophenol-containing "anti-fat nostrums" did not escape such attacks. For example, Rachel Lynn PALMER and Sarah GREENBERG wrote in their "*Facts and Frauds in Woman's Hygeine*" (1936):

"Yet the proprietary manufacturers, fully aware of these lethal results in medical practice, have the cold-blooded audacity to sell dinitrophenol nostrums for home use. Not only do the manufacturers fail to give any warning, but they assure their customers that the preparations are 'absolutely safe'. The sale of dinitrophenol products under these conditions can only be described as legal murder—for profit, of course."[39]

Other muckraking works, such as W. W. BAUER's "*Health, Hygeine and Hooey*" (1938), also vigorously denounced dinitrophenol as dangerous[40]. In her classic exposé, *American Chamber of Horrors: The Truth about Food and Drugs*" (1936), Ruth de FOREST LAMB also devoted several pages to a description of dinitrophenol nostrums and their dangers[41].

Another work, which was more of a popular treatise on reducing than a piece of muckraking literature, discussed dinitrophenol in a section entitled "DNP is TNT" in the following colorful language:

"Once again, I beg of you, lay off these dinitrophenol preparations. They are dangerous. Don't forget that this dinitrophenol was used in the manufacture of munitions during the World War, so it is just raring to go. Another bad actor, called dinitrocresol, is on the way up, so look out for that fellow also."[42]

Yet there was little that the Food and Drug Administration could do to control the problem. For one thing, cosmetics were not included under the 1906 Food and Drug Act if no therapeutic claims were made on the label, and there was a question as to whether or not obesity could be considered to be a disease[43]. In addition, Walter CAMPBELL, Chief of the Food and Drug Administration, complained in 1933 that his agency could not prohibit the sale to the public of drugs which are potentially dangerous and which should be used under medical supervision; they could only publish warnings about the dangers of such products[44]. In other words, the Food and Drug Administration did not have the authority to require that potent drugs be sold only on a prescription basis.

The FDA did, however, act against at least two of these nostrums containing nitrophenols. A product called "Slim", which consisted of one grain of dinitrophenol per tablet plus lactose as a diluent, claimed on the label to be the scientific way to reduce, a physician's prescription prepared under his personal

supervision, and a safe aid to weight reduction. In 1936, the FDA charged that these statements "were false and fraudulent representations regarding the curative or therapeutic effects of the article; and that they falsely and fraudulently represented that such products could be safely taken according to directions for reduction of superfluous weight." The manufacturer did not even bother to appear in court, so a default decree was entered. The only loss to the manufacturer, however, was the 26 bottles of Slim which had been seized[45]. In 1938, the FDA reported that it had prosecuted a dinitrocresol reducing agent for bearing false and fraudulent claims on the label. A plea of *nolo contendre* was entered and the court imposed a fine of $50.00[46].

When a new Food, Drug and Cosmetic Act was passed in 1938, the Food and Drug Administration was given the power to move more effectively against such products. Cosmetics were, of course, included under the FDA's jurisdiction under this Act, and, in addition, another provision of the Act defined a drug as misbranded if it was dangerous to health when used in the dosage or with the frequency or duration recommended in the label. Another provision of the Act required adequate directions for use and appropriate warnings against misuse. In an administrative decision, the FDA announced that potentially dangerous drugs (such as sulfanilamide) could not be distributed for unrestricted use by the public. CAMPBELL announced that drugs such as dinitrophenol, which were known to have produced harmful effects in many cases even when used under medical supervision, were going to be prosecuted by the FDA whenever found in interstate commerce, regardless of any label warnings that such products might carry. Apparently CAMPBELL's warning was sufficient to frighten such products off the market, since the FDA failed to find any traffic in dinitrophenol and dinitrocresol reducers after June of 1938 [47, 48]. The use of dinitrophenol and related compounds for treating obesity was essentially discontinued after the 1930's, and another chapter in the interesting history of this compound was closed.

The Mechanism of Action of Dinitrophenol

Scientists studying the effects of nitrophenols on metabolism naturally began to speculate on the mechanism by which these compounds stimulated oxidation. It was generally assumed that the increased heat production was a result of increased oxidation. A number of investigators began to study the action of nitrophenols on cell suspensions and on animal tissues *in vitro* (e.g., yeast cells, frog tissues), and they found that these substances produced an increase in oxygen consumption in isolated tissues as well as in whole animals[49, 50, 51]. Direct evidence was thus offered to further support the view that nitrophenols directly stimulated cellular respiration.

Evidence also began to accumulate in the early 1930's which suggested that dinitrophenols did not stimulate respiration by virtue of an ability to be reduced by the cell and reoxidized by oxygen. For example, R. H. DE MEIO and E. S. GUZMAN BARRON of the University of Chicago showed that dinitrophenol could not oxidize labile substances (the oxidation of which is readily catalyzed by reversible dyes or by hemin), and hence concluded that it does not act by direct oxidation of oxidizable substances, but "by combining with some of the substances acting as agents for the control of the speed of cellular oxidations, thus increasing the activity of the oxidizing enzymes."[50] M. E. KRAHL and G. H. A. CLOWES of the Lilly Research Laboratories and the Marine Biological Laboratory at Woods Hole agreed that dinitrophenols did not serve as an oxidation-reduction system, and suggested that it was more likely that they stimulated cellular respiration by accelerating one of the anaerobic processes concerned with the supply and activation of the substances destined to be oxidized[51]. TERADA and TAINTER also postulated that dinitrophenol acted as a catalyst to promote the oxidation of other substances[52].

CLOWES and KRAHL also demonstrated with sea urchin eggs that dinitrophenols blocked cell division, and thus inhibited growth, at the same time that they stimulated respiration[53]. The adverse effects of dinitrophenols on growing organisms had already been appreciated to a certain extent as they had been used to some degree as insecticides and herbicides. In 1892, the Bayer Company of Germany had introduced dinitro-o-cresol onto the market as an insecticide under the trade name of "Antinonnin".[54, 55, 56] Beginning about 1933, dinitrocresol came into widespread use as a herbicide in France, and by 1938 it was being used in the United States[57]. In 1934, a French scientist felt the need to issue a warning against the

indiscriminate use of dinitrophenol and dinitrocresol as insecticides and herbicides because of their toxicity[58]. Nitrophenols have continued to find use for these purposes up to the present time[59, 60]. Reports have appeared in the literature from time to time of fatalities among agricultural workers caused by dinitrophenol compounds[61].

Various experiments performed in the late 1930's and early 1940's suggested that dinitrophenol tended to interfere with energy-requiring reactions without interfering with oxidation. By the early 1940's the concept of oxidative phosphorylation had emerged, and the idea that dinitrophenol might possibly interfere with this process occurred to several investigators. KRAHL and CLOWES suggested in 1940 that nitrophenols acted on one or more of the oxidation-reduction or phosphorylating steps involved in the transfer of hydrogen in the respiratory chain[62]. KRAHL planned to follow up these possibilities, but became involved in war work and never returned to the project (personal communication). In 1943, Henry LARDY and Paul PHILLIPS of the University of Wisconsin postulated that dinitrophenol interferes with "the energy-coupling mechanism with the result that oxidation and glycolysis run rampant, while the energy is lost as heat rather than as work."[63] The focus of their studies, however, was sperm metabolism, and their observations on the mode of action of dinitrophenol were never followed up, although LARDY suggested again in 1945 (in an article co-authored with Conrad ELVEHJEM) that dinitrophenol might act by allowing oxidation to occur without phosphorylation[64]. Since dinitrophenol also appeared to increase the rate of hydrolysis of phosphocreatine and adenosinetriphosphate, it seemed possible that it might also block synthetic processes through increased hydrolysis of the phosphorylated compounds essential for synthesis[64, 65].

Fritz LIPMANN had met CLOWES at Woods Hole in the early 1940's and was fascinated by his work with KRAHL on nitrophenols[66]. LIPMANN was, of course, very much interested in oxidative phosphorylation, as evidenced by his classic review article on high energy phosphate bonds published in 1941[67], and it occurred to him that nitrophenols might interfere with this process. It was not until 1948, however, that he and W. F. LOOMIS demonstrated experimentally that dinitrophenol uncoupled oxidative phosphorylation[68]. It was thus clearly established that dinitrophenol and related compounds cause a rise in body temperature by preventing the energy of metabolism from being harnessed in phosphate compounds and causing it to be lost in the form of heat.

Conclusion

Dinitrophenol had proved to be a useful tool for biochemists in the study of metabolism. For example, it has been used to determine whether or not a metabolic process, such as peptide-bond formation, is dependent upon energy-rich phosphate compounds.

The mechanism of its action has been elucidated in more detail in the past quarter-century. The stimulation of respiration by dinitrophenol, for example, is due to the fact that uncoupled mitochondria respire at a high rate. Of course, it is not clear why this is the case and exactly how dinitrophenol causes uncoupling. Several theories have been offered, but the question is still unresolved[69]. Albert SZENT-GYÖRGI, commenting on the mechanism of action of dinitrophenol in 1957, called it "one of the most fascinating puzzles of contemporary biochemistry."[70]

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