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CHAPTER 9

Metabolites of shikimate and tryptophan pathways in coronavirus disease (COVID-19)

Abstract

Both Alzheimer disease (AD) and COVID-19 are age-dependent diseases with a high prevalence of obesity and diabetes. Proteomic and metabolomic profiling of sera from COVID-19 patients is discussed in this chapter. Some shikimate pathway-tryptophan metabolites are altered in the blood sera of severe COVID-19 versus healthy controls. Metabolites of bile acids increased in the sera of COVID-19 patients. In the serum metabolomics of severe COVID-19 patients versus healthy, serotonin was lower, —1.72 fold, and tryptophan was lower, —0.567 fold. The complete autopsy examinations may indicate that manifestations in different organs of COVID-19 patients can be caused by chronic and acute toxicity. This suggestion is consistent with data on serum metabolic profiling of COVID-19 patients.

Keywords: Complete autopsy cases; COVID-19; Serotonin; Serum metabolites; Shikimate pathway; Tryptophan.

9.1 Comparison of statistics for age-related diseases COVID-19 and Alzheimer disease

Both Alzheimer disease (AD) and COVID-19 are age-dependent diseases with a high prevalence of obesity and diabetes [2,372].

Approximately 10% (>200,000) of the population tested in Florida, United States, (>2 million people) are positive for the presence of coronavirus, which is related to coronavirus disease (COVID-19) https://floridahealthCOVID19.gov/ (data on July 7, 2020: positive 213,794, negative 2,055,400, total 2,269,194 or 9.42% of positive in total). About 2% (1.9%) of the ~10% positive individuals were critically ill and died (0.19% of Florida tested population). Total hospitalization in Florida with COVID-19 is 16,425, Florida resident deaths 3,841, and nonresident deaths 102. The current Florida population is 21.48 million people. Thus, 0.078% of the total Florida population was hospitalized with a diagnosis of COVID-19.

The rate of people who have been diagnosed with AD disease is 1.722% (5.7 million people in the US population of 331 million people). Thus, the rate of AD is 9.06-fold higher than the rate of COVID-19. The median time from AD dementia diagnosis to death was 3.8 years [373]. The 7.68% of COVID-19-positive patients have been hospitalized with severe symptoms.
Therefore, the prevalence of COVID-19 severe disease in Florida is essentially lower than the prevalence of another age-dependent disease, AD, that is considered to be noninfectious.

On March 8, 2020, the total positive cases in Florida were 491,884; negative 3,260,914; death 7157. Positive cases by exposure source on March 8, 2020, indicated as traveled 3737; contact with confirmed case 133,732; travel and contact with confirmed case 3756; under investigation 323,681; for a total of 491,884. Thus, the sources for majority of positivity are not known.

Table 9.1 includes the data on positivity for the virus related to COVID-19 in different zip codes, mainly in Miami-Dade, Florida. These data show no direct relation between the population density and COVID-19 virus positivity. Particularly, the Miami-Dade zip code 33141 with population density of 18.27 subjects per square mile showed 0.9% positivity per population, while zip code 33125 with a population density 13.83 showed 4.5% positivity (Table 9.1). Of note, no data on hospitalization of COVID-19 positive cases are available for each zip code to be reviewed and analyzed.

Density of population does not of itself determine the ease with which infection spreads through a population. Problems tend to arise primarily when populations become so dense as to cause overcrowding. Overcrowding is often associated with decreases in quality of living conditions and sanitation, so the rate of agent transmission is typically very high in such areas. Thus, overcrowded cities or densely populated areas of cities can potentially serve as breeding grounds for infectious agents, which may facilitate their evolution, particularly in the case of viruses and bacteria. Rapid cycling between humans and other hosts, such as rats or mice, can result in the emergence of new strains capable of causing serious disease (https://www.britannica.com/science/infectious-disease/Population-density).

The recent Centers for Disease Control and Prevention (CDC) data indicate the virus linked to COVID-19 has been found in untreated wastewater (https://www.cdc.gov/coronavirus/2019-ncov/community/sanitation-wastewater-workers.html). The leakage of untreated wastewater happened previously during some storms, hurricanes, and consequent flooding in Miami, Florida, and New York City, NY.

The CDC estimates that influenza was associated with more than 35.5 million illnesses, more than 16.5 million medical visits, 490,600 hospitalizations, and 34,200 deaths during the 2018–19 influenza season. This burden was similar to the estimated burden during the 2012–13 influenza season (Centers for Disease Control and Prevention. Estimated influenza illnesses and hospitalizations averted by influenza vaccination, United States, 2012–13 influenza season. MMWR Morb Mortal Wkly Rep 2013; 62(49): 997–1000).
Table 9.1 Coronavirus (COVID-19)-related positivity in Miami-Dade County and some other Florida Counties (by zip codes) in the period of spike in positivity (July 6–7, 2020; August 3, 2020; and then 1/29/2021). Cases data are from Florida’s COVID-19 Data and Surveillance Dashboard. Florida Department of Health, Division of Disease Control and Health Protection.

| Zip code | City | Population | Land area (sq mi) | Number of positive cases | Percent positive per zip code population | Number positive 1/29/2021 |
|----------|------|------------|-------------------|-------------------------|-----------------------------------------|---------------------------|
| 33154    | Surfside | 13,971     | 1.78 (7,840<sup>a</sup>) | 134                     | 0.959                                   | 1522 (10.89%<sup>b</sup>) |
| 33154    |       |            |                   | 313 (8/3<sup>c</sup>)   | 2.24                                    |                           |
| 33031    | Homestead | 5,859     | 21.37 (274)       | 56                      | 0.95                                    | 649 (11.07%)              |
| 33032    | Homestead | 34,088    | 18.8 (1,813)      | 655                     | 1.9                                     | 6758 (19.82%)             |
| 33032    |       |            |                   | 1889 (8/3)              | 5.5                                     |                           |
| 33033    | Homestead | 49,028    | 16.8 (2,918)      | 1093                    | 2.2                                     | 7557 (15.4%)              |
| 33033    |       |            |                   | 2709 (8/3)              | 5.52                                    |                           |
| 33034    | Homestead | 18,613    | 279.747 (67)      | 727                     | 3.9                                     | 2527 (13.57%)             |
| 33035    | Homestead | 13,497    | 20.778 (652)      | 249                     | 1.84                                    | 1589 (11.77%)             |
| 33035    |       |            |                   | 548 (8/3)               | 4.06                                    |                           |
| 33157    | Cutler Bay | 63,226    | 14.82 (4,266)     | 1272                    | 2.01                                    | 8373 (13.24%)             |
| 34142    | Immokalee, Collier County | 27,304 | 589.251 (46.3) | 1579 | 5.8 | 3227 (11.8%) |
| 33139    | Miami Beach | 38,613    | 2.695 (14,354)    | 559                     | 1.44                                    | 5061 (13.1%)              |
|          | Lincoln Road |   |                   | 1189 (8/3)              | 3.07                                    |                           |
| 33140    | Miami Beach flooding zone | 21,210 | 3.05 (6,954)   | 539                     | 2.54                                    | 3059 (14.4%)              |
| 33141    | Miami Beach, North Bay Village | 35,249 | 2.32 (15,222) | 388 | 1.1 | 3752 (10.6%) |
| 33179    | North Miami Beach, Miami Gardens | 41,332 | 5.03 (8,211) | 710 | 1.7 | 4715 (11.4%) |
| 33125    | Miami, Liberty City | 52,677 | 3.9 (13,506) | 2443 | 4.6 | 10232 (19.4%) |
| 33125    |       |            |                   | 4877 (8/3)              | 9.27                                    |                           |
| 33142    | Miami, Hialeah | 52,606 | 7.07 (7,436) | 1737 | 3.3 | 7978 (15.1%) |
| 33142    |       |            |                   | 4142 (8/3)              | 7.87                                    |                           |
| 33458    | Jupiter, Palm Beach County | 49,396 | 21.6 (2,148) | 693 | 1.4 | 3478 (7.04%) |
| 33401    | West Palm Beach, Palm Beach County | 24,879 | 5.2 (4,784) | 454 | 1.82 | 2945 (11.84%) |
| 33009    | Broward County Hallandale | 39,341 | 5.09 (7,729) | 390 | 0.99 | 3591 (9.13%) |
| 33180    | Aventura | 30,840 | 3.4 (9,070) | 918 (8/3) | 2.98 | 3704 (12%) |
| 33156    | Dade Pinecrest, Kendall | 31,315 | 13.57 (2,307) | | | |
| 33143    | Dade Kendal | 31,404 | 7.89 (3,980) | | | |

Numbers of positives (cases) by zip code are from https://experience.arcgis.com/experience/96dd742462124fa0b38dded9b25e429. The population of the zip code is based on Census 2010 (https://www.zip-codes.com/zip-code/33154/zip-code and https://www.unitedstateszipcodes.org/). Of note, the population likely increased essentially in some zip codes from Census 2010 to January 2021. Table 9.1 shows that COVID-19 virus positivity is not correlated with population density in Florida.

<sup>a</sup>Number in parentheses (land area): density of population per sq. miles.

<sup>b</sup>Percent positive of population is in the zip code on 1/29/2021.

<sup>c</sup>8/3 data on August 3, 2020.
9.2 Proteomic and metabolomic profiling of sera from COVID-19 patients and further discussion

Shen et al. performed proteomic and metabolomic profiling of the blood sera from 46 COVID-19 and 53 control individuals [372]. The author’s team procured serum samples from 65 COVID-19 patients who visited Taizhou Hospital, China, from January to March 2020. Drugs are not used in the following analysis. In the serum metabolomics of severe COVID-19 patients versus healthy the following metabolites were significantly altered: benzoate was 6.3-fold higher (P value 2.7E-25), serotonin was lower −1.7258 fold (P value 9.0669E−07), tryptophan was lower −0.567 fold (P value 5.08E−05), indoleacetate (nonsevere vs. healthy), a tryptamine metabolite was lower at −0.39 fold (P value .003), quinolinate was higher 1.44-fold (P value .00156), quinate was lower −0.4906 fold (P value .0083), and thyroxine was lower −0.67 fold (P value 2.14E−06) (Fig. 9.1).

In comparison of severe versus nonsevere COVID-19 cases, the blood serum quinolinate was higher 1.488-fold (P value .00178), thyroxine was lower, −0.4868-fold (P value .00208), taurochenodeoxycholic acid 3-sulfate was higher 2.21-fold (P value 2.48041E−05), and taourursodeoxycholic acid sulfate was higher 3.178-fold (P value 2.736 E−05).

**Figure 9.1** Activities of blood serum shikimate pathway-tryptophan metabolites altered in severe COVID-19 versus healthy controls.
Choline was lower in severe COVID-19 patients than in healthy controls at −0.47 fold (P value 6.216 E−06) and in nonsevere COVID-19 versus healthy choline −0.46 fold (P value 1.219 E−07). Choline metabolite betaine [374] was lower in severe COVID-19 than in the healthy group at −0.42 fold (P value .0002) [372]. Choline deficiency can cause disorders in many bodily systems, including liver, muscle, and lymphocytes in humans [375]. Phosphocholine, a human metabolite phosphate of choline, was higher in severe COVID-19 group than in other groups. Cholinesterase inhibitors, which function by inhibiting cholinesterase from hydrolyzing acetylcholine into its components of acetate and choline (Fig. 9.2), allowing for an increase in the availability and duration of action of acetylcholine in neuromuscular junctions, may present in the COVID-19 patients.

Uchida and Yamashita showed that enzyme choline kinase catalyzes formation of phosphocholine from choline and phosphate of ATP. Spermine and spermidine stimulated this enzyme by decreasing the apparent Km for ATP and increasing Vmax [376]. Acetylcholine was also stimulatory.

Taurocholic acid 3-sulfate (TCA3S) is a metabolite of the conjugated bile acid taurocholic acid (a taurine-conjugated form of cholic acid). Plasma levels of TCA3S

![Figure 9.2](image-url)  
**Figure 9.2** Cholinesterase catalyzes hydrolysis of acetylcholine into choline and betaine, and benzoylcholine into choline and benzoic acid, while benzalkonium degrades into benzoic acid and inhibits cholinesterase. Tryptamine inhibits cholinesterase and affects hydrolysis by cholinesterase.
are elevated in wild-type and Sortilin 1 (Sort1) knockout mice at 6 h following bile duct ligation (BDL) and are further elevated in Sort1 knockout mice at 24 h post-BDL [377].

Bile acids are produced in the liver and excreted into the intestine, where their main function is to participate in lipid digestion. Ursodeoxycholic acid and tauroursodeoxycholic acid have shown antiapoptotic, anti-inflammatory, and antioxidant effects in various models of neurodegenerative diseases [378]. Taurochenodeoxycholic acid 3-sulfate and taouroursodeoxycholic acid sulfate (1) are statistically significantly higher (4.34-fold and 6.34-fold, respectively) in sera of non-COVID-19 patients than in controls.

Quinate synthesizes through a side branch of the shikimate pathway. Quinolinic acid (quinolinate) is a downstream product of the kynurenine pathway, which metabolizes the amino acid tryptophan. Quinolinic acid has a potent neurotoxic effect [379].

Tauroursodeoxycholic acid is the more hydrophilic form of ursodeoxycholic acid, which is the more abundant naturally produced bile acid in humans. Tauroursodeoxycholic acid, on the other hand, is produced abundantly in bears and has been used for centuries as a natural remedy in some Asian countries. It is approved in Italy and Turkey for the treatment of cholesterol gallstones and is an investigational drug in China, the Unites States, and Italy. Tauroursodeoxycholic acid is being investigated for use in several conditions such as primary biliary cirrhosis, insulin resistance, amyloidosis, cystic fibrosis, cholestasis, and amyotrophic lateral sclerosis.

A report of the World Health Organization “Benzoic acid and sodium benzoate,” 2000, (Concise International Chemical Assessment Document 26) concluded that the acute toxicity of benzoic acid and sodium benzoate in humans is low [380]. However, both substances are known to cause contact dermatitis (pseudoallergy). In patients with urticaria or asthma, an exacerbation of the symptoms was observed after testing (oral provocation test or patch tests), whereas this effect is unusual in healthy subjects [380]. Their antimicrobial properties are used for different applications, such as food preservation. Benzoates applied dermally can penetrate through the skin.

Paley discussed the activities of the human fecal metabolite benzoate in a recent article [16]. Particularly, Hoffmann and Grond, 2004, demonstrated that in *Streptomyces* sp., benzoate forms directly from shikimate [381], a precursor for chorismate, which is a precursor for the aromatic amino acids phenylalanine, tryptophan, and tyrosine. Benzoate is widely used by the food industry to prevent spoilage and to inhibit the growth of pathogenic microorganisms [382]. Sodium benzoate therapy improved symptomatology of patients with schizophrenia [383]. Zinc benzoate, commonly used in food and feed additives as a preservative and source of zinc (Zinc Benzoate CAS: 553-72-0, Silver Fern
Chemical Inc.), inhibits MAO-A activity [384]. Zinc benzoate reversibly and competitively inhibited MAO-A activity in a dose-dependent manner. Being an MAO inhibitor, zinc benzoate can act as a protoxin since it can lead to accumulation of toxic biogenic amines. Zinc benzoate is an environmental contaminant derived from polystyrene. No data is available in respect of MAO inhibitory activity for sodium benzoate or benzoic acid. Thus, further research is needed to answer the question whether any salts of benzoic acid can inhibit MAO or only specific salts of benzoic acid such as zinc benzoate are able to inhibit this enzyme.

SDS (formerly known as MSDS) informs about benzoic acid uses and safety (https://www.msdsonline.com/2015/02/16/benzoic-acid-uses-and-safety/). Specifically, benzoic acid is a compound naturally found in many plants and is an important precursor for the synthesis of many other organic substances. Benzoic acid is most commonly found in industrial settings to manufacture a wide variety of products such as perfumes, dyes, topical medications, and insect repellents. Benzoic acid's salt (sodium benzoate) is commonly used as a pH adjustor and preservative in food, preventing the growth of microbes to keep food safe. It works by changing the internal pH of microorganisms to an acidic state that is incompatible with their growth and survival. Immediately or shortly after exposure to benzoic acid, the following health effects can occur: eye damage, irritation of the skin, resulting in a rash, redness, and/or a burning feeling, irritation to the nose, throat, and lungs if inhaled, which may cause coughing, wheezing, and/or shortness of breath.

I suggest that bacteria and other microorganisms may develop tolerance/resistance to benzoic acid/benzoate. Creamer et al. reported that *Escherichia coli* K-12 W3110 grows in the presence of membrane-permeant organic acids that can depress cytoplasmic pH and accumulate in the cytoplasm. The authors conducted experimental evolution by daily diluting cultures in increasing concentrations of benzoic acid (up to 20 mM) buffered at external pH 6.5, a pH at which permeant acids concentrate in the cytoplasm. By 2000 generations, clones isolated from evolving populations showed increasing tolerance to benzoate but were sensitive to chloramphenicol and tetracycline. Sixteen clones grew to stationary phase in 20 mM benzoate, whereas the ancestral strain W3110 peaked and declined. Similar growth occurred in 10 mM salicylate. Benzoate-evolved strains grew like W3110 in the absence of benzoate, in media buffered at pH 4.8, pH 7.0, or pH 9.0, or in 20 mM acetate or sorbate at pH 6.5. Genomes of 16 strains revealed over 100 mutations, including single-nucleotide polymorphisms, large deletions, and insertion knockouts. Most strains acquired deletions in the benzoate-induced multiple antibiotic resistance regulon or in associated regulators [385].
Therefore, bacteria can develop tolerance to benzoate. Moreover, benzoate can become a selective agent in the human microbiome. Furthermore, such microbial selection can lead to dysbiosis, which is implicated in a number of medical conditions including AD [178]. Microorganisms can also use benzoate as a source of carbon. Cinar, 2004, studied the response of a mixed microbial culture to different feed compositions, that is, containing benzoate and pyruvate as sole carbon sources at different levels, in a chemostat with a 48-h hydraulic residence time under cyclic aerobic and anoxic (denitrifying) conditions. The cyclic bacterial culture was well adapted to different feed compositions as evidenced by the lack of accumulation of benzoate or pyruvate in the chemostat [386].

Further analysis of fecal samples from COVID-19 patients can reveal dysbiosis in these patients (Fig. 9.1).

Lennerz et al. reported that anthranilic acid, a tryptophan metabolite, exhibited a robust rise, while acetylglycine dropped through a randomized, controlled, cross-over study of 14 overweight subjects. In this study, serial blood samples were collected following an oral glucose challenge, in the presence or absence of sodium benzoate. Outcome measurements included glucose, insulin, glucagon, as well as temporal mass spectrometry-based metabolic profiles [387]. Genetic and nutritional studies with fungus *Neurospora crassa* indicate that the first enzyme specifically involved in tryptophan biosynthesis catalyzes the formation of anthranilic acid [388]. Anthranilic acid, which is involved in tryptophan metabolism in both humans and bacteria, could reflect an influence on tryptophan metabolism in either the subjects themselves or their gut microbial species, or both. It is also notable that anthranilic acid and benzoate differ by a single amine group, so the anthranilic acid may come directly from the ingested benzoate itself, either through metabolism by the subjects or their microbiota, though there is no annotated enzyme that catalyzes this reaction. Anthranilic acid accumulates in the setting of renal failure, and one cell culture study suggested that it might promote renal failure through adverse effects on mesangial cells. In a separate study, treatment of cultured neurons and glial cells with anthranilic acid altered NAD+ levels and caused cytotoxicity. Although this study shows that benzoate does not have an acute, adverse effect on glucose homeostasis, future studies will be necessary to explore the metabolic impact of chronic benzoate exposure [387].

Wild-type *Streptomyces maritimus* produces benzoate via a plant-like β-oxidation pathway and can assimilate various carbon sources for benzoate production [389]. Presence of *Streptomyces* has been shown in human gut microbiome [390] and in human lung [391].

Raposa et al. demonstrated that sodium benzoate (from low to high doses) dose-dependently silenced MAPK8 (mitogen-activated protein kinase 8) expression \((P = .004 \text{ to } P = .002)\) [392].
Khoshnoud et al. [393] investigated the effects of oral administration of different concentrations of sodium benzoate (0.56, 1.125, and 2.25 mg/mL) for 4 weeks, on the learning and memory performance tests, and also the levels of malondialdehyde (MDA), reduced glutathione (GSH), and acetylcholinesterase activity (AChE) in the mouse brain. The results showed that sodium benzoate significantly impaired memory and motor coordination. Moreover, sodium benzoate decreased GSH and increased the MDA level in the brain significantly \( (P < .001) \), and nonsignificant alteration was observed in the AChE activity. These findings suggest that short-term consumption of sodium benzoate can impair memory performance and increased brain oxidative stress in mice [393].

Furthermore, benzoic acid is formed by the enzymatic hydrolysis of benzoylcholine with cholinesterase [394] (Fig. 9.2). Akcasu et al. described the pharmacology of benzoylcholine in 1952 [395]. Benzoylcholine is known to be broken down in the body into benzoic acid and choline. Benzoylcholine has a direct stimulant action on gut and heart; this action is unaffected by atropine [395]. Benzoylcholine is a neuromuscular blocking agent in rabbits and is relatively nontoxic \( (LD_{50} \text{ in rabbits: 150 mg/kg}) \). It produces neuromuscular block in the rat diaphragm and cat gastrocnemius and paralysis in chicks. On smooth muscle, benzoylcholine appears to exert at least three distinct actions. In small doses in the trachea preparations, it potentiates the acetylcholine response, possibly by inhibition of the cholinesterase; in medium doses in all preparations, it blocks the acetylcholine response, possibly by attaching itself to the same receptors; and in larger doses, it stimulates most forms of smooth muscle, in part through a direct stimulant action [395]. Benzoylcholine has been widely used in enzyme studies as a substrate for pseudo-cholinesterase. It is freely soluble in water. Erdos and colleagues reported in Science (1957) that tryptamine accelerates the enzymatic hydrolysis of benzoylcholine by plasma cholinesterase [396] (Fig. 9.2). A series of derivatives of benzoylcholine has been prepared with substituents in the benzene ring, and the rate of hydrolysis of these compounds with cholinesterase of horse serum has been determined and reported in 1953 by Ormerod [397]. In 2006, the catalytic properties of rat butyrylcholinesterase with benzoylcholine and N-alkyl derivatives of benzoylcholine used as substrates were reported by Hrabovska et al. [398]. Docking studies showed that long-chain substrates were not optimally oriented in the active site for catalysis, thus explaining the slow rate of hydrolysis [398]. The simultaneous increases in benzoate and phosphocholine and choline decrease in sera of severe COVID-19 patients can, presumably, derive from hydrolysis of the candidate molecule such as benzoylcholine or its derivative by plasma cholinesterase.

Another candidate molecule is benzalkonium chloride (Fig. 9.2), also known as alkyl dimethyl benzyl ammonium chloride, which is classed as an antiseptic active ingredient
by the United States Food and Drug Administration and can be oxidized to benzoic acid [399]. Jaganathan and Boopathy demonstrated in 2000 [400] the distinct effect of benzalkonium chloride on the esterase and aryl acylamidase activities of butyrylcholinesterase. Acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) from vertebrates, other than their predominant acetylcholine hydrolase (esterase) activity, display a genuine aryl acylamidase activity (AAA) capable of hydrolyzing the synthetic substrate o-nitroacetanilide to o-nitroaniline. Benzalkonium chloride (BAC), a cationic detergent widely used as a preservative in pharmaceutical preparations, has been shown to distinctly modulate the esterase and AAA activities of BChEs. The detergent BAC was able to inhibit the esterase activity of human serum and horse serum BChEs and AChEs from fish electric eel and human erythrocyte. BAC binds to the active site of ChEs. BAC was able to profoundly activate the AAA activity of human serum and horse serum BChEs [400]. Tryptamine is able to inhibit both AAA and ChE activities [246]. Swiercz et al. demonstrated in 2008 the pulmonary irritation after inhalation exposure to benzalkonium chloride in rats [401]. BAC may be classified to class I acute inhalation toxicity. It showed a strong inflammatory and irritant activity on the lungs after 6-h inhalation [401]. The cases of intentional and accidental poisoning of humans with benzalkonium chloride were described. In 2018, a forensic autopsy case of an elderly man who ingested an unknown amount of germicidal disinfectant containing 50% benzalkonium chloride (BZK) was reported. He survived for 18 days after BZK ingestion and then died because of pneumonia [402]. Hitosugi et al. reported in 1998 a case of fatal benzalkonium chloride poisoning [403]. In this report, five elderly persons with senile dementia accidentally ingested Hoesmin, a 10% aqueous solution of benzalkonium chloride (BAC). The condition of one patient, an 84-year-old woman whose lips and oral cavity became erythematous, gradually deteriorated. Although gastric lavage was performed, the patient died 3 h after ingestion of Hoesmin. Autopsy revealed corrosive changes of the mucosal surfaces of the tongue, pharynx, larynx, esophagus, and stomach, which may have come in contact with BAC. In addition, BAC was detected in the serum. The authors conclude that the patient died of BAC poisoning [403]. Wilson and Burr reported in 1975 a case of benzalkonium chloride poisoning in infant twins [404]. In this case, infant twins sustained severe circumoral and pharyngeal burns from a concentrated solution of benzalkonium (Zephiran) chloride prescribed for treatment of candidiasis. This report emphasizes the unnecessary hazard accompanying use of a potentially toxic drug, especially when prepared in error by the pharmacist. Risks from use of a prescription drug for other than the intended patient are also highlighted by this episode of poisoning [404]. A news report published on July 10, 2018, (https://nypost.com/2018/07/10/japanese-nurse-admits-she-killed-over-20-elderly-patients/) informed that a Japanese nurse admitted
to killing at least 20 elderly patients. Ayumi Kuboki allegedly poisoned the patients by lacing their intravenous (IV) drips with toxic antiseptic chemicals at specific times, Japanese newspaper the Asahi Shimbun reported. Police believe the caregiver put cleaning product containing benzalkonium chloride into patients' IVs, which left patients dead within hours. The chemical, an antibacterial that is readily available within hospitals, was found in the patient's body and his IV bag. Ayumi Kuboki, who was arrested in July on suspicion of killing at least three and possibly a fourth patient, was ordered to undergo a psychiatric evaluation for 3 months from September until the end of November, Fuji TV reported. Prosecutors said the evaluation had found her mentally fit to stand trial.

Pereira and Tagkopoulos published a review in 2019 on benzalkonium chloride uses, regulatory status, and microbial resistance [405]. BACs were reported for the first time in 1935 by Gerhard Domagk, gaining the market as zephiran chlorides, and were marketed as promising and superior disinfectants and antiseptics. In 1947, the first product containing BACs was registered with the Environmental Protection Agency in the United States. Since then, they have been used in a wide variety of products, both prescription and over the counter. Applications range from domestic to agricultural, industrial, and clinical [405]. BACs have been detected in food samples, with a maximum at 14.4 mg/kg [405]. High-performance liquid chromatography and gas chromatography–mass spectrometry analysis was used to study BAC degradation pathway. It was shown that during BAC biodegradation by bacterium Aeromonas hydrophila sp., formation of benzyldimethylamine, benzylmethylamine, benzylamine, benzoaldehyde, and benzoic acid occurred [399]. Biodegradation of benzalkonium chlorides singly and in mixtures by a Pseudomonas sp. isolated from returned activated sludge was reported by Khan et al. in 2015 [406]. At least 40 outbreaks have been attributed to infection by disinfectant- and antibiotic-resistant pathogens such as Pseudomonas aeruginosa. Kim et al. reported in 2018 the genomic and transcriptomic insights into how bacteria withstand high concentrations of benzalkonium chloride [407]. Gene expression changes in BAC-adapted P. aeruginosa were revealed. Particularly, overexpression of biogenic amine spermidine synthesis genes was detected in BAC-adapted bacteria [407]. Both spermidine (twofold) and N(1)-acetylspermidine (2.6-fold) are higher in sera of non-COVID patients versus healthy group. Seguin et al. reported in 2019 [408] that while there exists a large and growing body of literature concerning the toxicology of BACs, information on the metabolism of BACs in mammalian species is still lacking. Single-dose intravenous injection of BACs to rats (7 mg/kg) led to a wide distribution of these compounds in various tissues (levels in μg/g of tissue shown in the parentheses), with the highest level observed in the kidney (50.5), followed by lung and spleen (15.4 each), serum (1.2), liver (0.9), and brain (0.2) after 30 min of administration. When BACs were administered orally (250 mg/kg), the levels of BAC reached their highest concentrations for the majority of the tissues after 24 h (2 h for liver), with the level in kidney (5.25) > lung (2.75) >
liver (0.72) > blood (0.34) (not determined in brain). Therefore, BACs are orally bioavailable and distribute broadly throughout tissues in vivo. However, the mechanisms of metabolism and disposition of BACs in humans have not been studied, and this represents a barrier to our understanding of the systemic toxicology of BACs [408]. A few studies have reported the microbial degradation of BAC by several pure cultures (Pseudomonas nitroreducens, Aeromonas hydrophila, and Bacillus niabensis) to benzyldimethylamine by dealkylating amine oxidase and related enzymes [409]. Metagenomic analysis of a river sediment microbial community by Oh et al. revealed that BAC exposure selected for a low-diversity community, dominated by several members of the Pseudomonas genus that quickly degraded BACs [410]. Metatranscriptomic analysis of this microbial community during a complete feeding cycle with BACs as the sole carbon and energy source was conducted under aerobic conditions. P. nitroreducens isolates from the BAC-fed bioreactor can grow on BACs as a sole carbon and energy source by dealkylating the parent molecules and producing stoichiometric quantities of benzyldimethylamine (BDMA) as a dead-end product. The genes involved in BAC dealkylation and β-oxidation based on bioinformatics and/or genetic analyses show BDMA production from BAC by P. putida and P. entomophila [410]. Phylogenetic affiliation of gene transcripts related to the three known benzoate degradation pathways was demonstrated mainly for Pseudomonas species [410]. The metatranscriptomic profiles showed increased expression of genes predicted to be associated with the biodegradation of benzoate, the by-product of BDMA metabolism, by enzyme such as benzoate dioxygenase (benABC). These results indicated that the benzyl compounds produced from the dealkylation of BACs by P. nitroreducens were predominantly metabolized by P. putida and P. entomophila [410]. The control of synthesis of the five enzymes responsible for the conversion of D(-)-mandelate to benzoate by Pseudomonas putida was investigated [411]. Benzoate is converted to catechol in this pathway. The members of the class of catechols are significantly lower in COVID-19 sera compared to controls. For instance, 4-ethylcatechol sulfate was $-4.92$-fold lower in severe COVID-19 cases compared to controls. Catecholamine metabolite vanillylmandelate was $-0.407$-fold lower in nonsevere COVID-19 compared to healthy subjects. Another benzoate metabolite hippurate is also statistically significantly decreased in severe COVID-19 versus healthy group: hippurate ($-1.466$-fold), 3-hydroxyhippurate ($-2.23$-fold), 2-hydroxyhippurate (salicylurate) ($-1.669$-fold). The 3-hydroxyhippurate ($-1.31$-fold) and hippurate ($-1.0344$-fold) are decreased in non-COVID-19 patients versus healthy group [372].

Pallister et al. reported in 2017 [412] that an increasing hippurate trend was associated with reduced odds of having metabolic syndrome. Thus, the data on hippurate serum content are consistent with increased odds of having metabolic syndrome in COVID-19 patients.

Chen et al. demonstrated that sodium benzoate exposure downregulates the expression of tyrosine hydroxylase and dopamine transporter in dopaminergic neurons in
developing zebrafish [413]. The results suggest that sodium benzoate exposure can cause significantly decreased survival rates of zebrafish embryos in a time- and dose-dependent manner and in decreased locomotor activity of zebrafish larvae [413].

Piper and Piper reported in a review that benzoate can react with the ascorbic acid in drinks to produce the carcinogen benzene [414]. A few children develop an allergy to this additive. As a competitive inhibitor of \( \text{D-} \)amino acid oxidase, benzoate [415] can also influence neurotransmission and cognitive functioning. Model organism and cell culture studies have raised some issues. Benzoate has been found to exert teratogenic and neurotoxic effects on zebrafish embryos. In addition, benzoate and sorbate are reported to cause chromosome aberrations in cultured human lymphocytes, as well as to be potently mutagenic toward the mitochondrial DNA in aerobic yeast cells. Whether the substantial human consumption of these compounds could significantly increase levels of such damages in man is still unclear [414]. Benzoate rapidly traverses the blood—brain barrier and is now attracting increased attention as an agent for the treatment of certain brain disorders [414], partly because it presents the advantages of a ready oral administration and an existing approval for the treatment of urea cycle disorders (UCD) [416]. Cinnamon—the most consumed spice worldwide—is also of interest in this regard, since the oral feeding of cinnamon (\textit{Cinnamomum verum}) powder is known to generate benzoate in the blood and brain of mice [414].

\( \text{D-} \)amino acid oxidase is the prototype of the flavin adenine dinucleotide (FAD)–dependent oxidases. It catalyzes the oxidation of \( \text{D-} \)amino acids to the corresponding \( \text{D-} \)ketoacids. The reducing equivalents are transferred to molecular oxygen with production of hydrogen peroxide. The crystal structure of the complex of \( \text{D-} \)amino acid oxidase with benzoate, a competitive inhibitor of the enzyme, has been solved by Mattevi et al. [417].

Fagnant et al. evaluated virus survival over time on ViroCap filters. The filters were seeded with poliovirus (PV) type 1 (PV1) and/or bacterium \textit{Escherichia coli} virus MS2 and then dosed with preservatives or antibiotics prior to storage and elution. These filters were stored at various temperatures and time periods, and then eluted for PV1 and MS2 recovery quantification. Filters dosed with the preservative combination of 2% sodium benzoate and 0.2% calcium propionate had increased virus survival over time when stored at 25°C, compared to samples stored at 25°C with no preservatives [418].

The benzoate increase was highest among the serum metabolites altered in Chinese severe COVID-19 patients compared to healthy individuals [372]. Serotonin was lower in sera of severe COVID-19 patients compared to healthy controls [372] and also in aging, depression, and AD patients [105].

9.3 Postmortem studies of COVID-19 patients in different countries

Most patients with COVID-19 are asymptomatic or experience only mild symptoms, including fever, dry cough, and shortness of breath. However, some individuals
deteriorate rapidly and develop acute respiratory distress syndrome (ARDS). The most common histopathologic correlate of ARDS is diffuse alveolar damage (DAD), characterized by hyaline membrane formation in the alveoli in the acute stage, and interstitial widening by edema and fibroblast proliferation in the organizing stage. DAD has a long list of potential etiologies, including infection, vaping-associated pulmonary injury, oxygen toxicity, drug toxicity, toxic inhalants or ingestants, shock, severe trauma, sepsis, irradiation, and acute exacerbations of usual interstitial pneumonia [419].

Schaller et al. in a recent report of examinations conducted in Germany, state that “because there are still insufficient data on cause of death, we describe postmortem examinations in a case series of patients with COVID-19” [420]. Between April 4 and April 19, 2020, the serial postmortem examinations were conducted in patients with proven severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection who died at the University Medical Center Augsburg (Germany). Autopsies were conducted according to published best practice. Specimens from lung, heart, liver, spleen, kidney, brain, pleural effusion, and cerebrospinal fluid (CSF) were assessed. Postmortem nasopharyngeal, tracheal, bronchial swabs, pleural effusion, and CSF were tested for SARS-CoV-2 by reverse transcriptase—polymerase chain reaction. In this postmortem evaluation of 10 patients with COVID-19, acute and organizing DAD and SARS-CoV-2 persistence in the respiratory tract were the predominant histopathologic findings and constituted the leading cause of death in patients with and without invasive ventilation. Periportal liver lymphocyte infiltration was considered unspecific inflammation. Whether myoepicardial alterations represented systemic inflammation or early myocarditis is unclear; criteria for true myocarditis were not met. Central nervous system involvement by COVID-19 could not be detected. This study has limitations, including the small number of cases from a single center and missing proof of direct viral organ infection [420].

Pulmonary postmortem findings in a series of COVID-19 cases were revealed in a northern Italy two-center descriptive study [421]. The predominant pattern of lung lesions in patients with COVID-19 patients is DAD, as described in patients infected with severe acute respiratory syndrome and Middle East respiratory syndrome coronaviruses. Hyaline membrane formation and pneumocyte atypical hyperplasia are frequent. Importantly, the presence of platelet—fibrin thrombi in small arterial vessels is consistent with coagulopathy, which appears to be common in patients with COVID-19 [421].

In Chinese reports, COVID-19, caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection, is associated with coagulopathy causing venous and arterial thrombosis [422,423]. Recent data from the pandemic epicenter in Wuhan, China, reported neurologic complications in 36% of 214 patients with COVID-19; acute cerebrovascular disease (mainly ischemic stroke) was more common among 88 patients with severe COVID-19 than those with nonsevere disease (5.7% vs. 0.8%) [424].

However, the mechanisms, phenotype, and optimal management of ischemic stroke associated with COVID-19 remain uncertain.
Researchers in London, United Kingdom, described the demographic, clinical, radiologic, and laboratory characteristics of six consecutive patients assessed between and April 1 and 16, 2020, at the National Hospital for Neurology and Neurosurgery, Queen Square, London, UK, with acute ischemic stroke and COVID-19 (confirmed by reverse-transcriptase PCR (RT-PCR)). All six patients had large vessel occlusion with markedly elevated D-dimer levels ($\geq 1000 \mu\text{g/L}$). Three patients had multiterritory infarcts, two had concurrent venous thrombosis, and in two, ischemic strokes occurred despite therapeutic anticoagulation [425].

In New Orleans, USA, autopsies were performed on 10 African American decedents aged 44–78 years with cause of death attributed to COVID-19, reflective of the dominant demographic of deaths following COVID-19 diagnosis [426]. Important findings include the presence of thrombosis and microangiopathy in the small vessels and capillaries of the lungs, with associated hemorrhage, that significantly contributed to death. Features of DAD, including hyaline membranes, were present, even in patients who had not been ventilated. Cardiac findings included individual cell necrosis without lymphocytic myocarditis. There was no evidence of secondary pulmonary infection by microorganisms [426].

Solomon et al. reported that neurologic symptoms, including headache, altered mental status, and anosmia, occur in many patients with COVID-19. The neuropathologic findings were from autopsies of 18 consecutive patients with SARS-CoV-2 infection who died in a single teaching hospital between April 14 and April 29, 2020, in Massachusetts, USA [427]. All the patients had nasopharyngeal swab samples that were positive for SARS-CoV-2 on qualitative reverse transcriptase–polymerase chain reaction (RT-PCR) assays. Histopathologic examination of brain specimens obtained from 18 patients who died 0–32 days after the onset of symptoms of COVID-19 showed only hypoxic changes and did not show encephalitis or other specific brain changes referable to the virus. The virus was detected at low levels in six brain sections obtained from five patients; these levels were not consistently related to the interval from the onset of symptoms to death. Positive tests may have been due to in situ virions or viral RNA from blood [427].

The autopsy findings of 21 COVID-19 patients hospitalized at the University Hospital Basel and at the Cantonal Hospital Baselland, Switzerland, were reported by Menter et al. [428]. The primary cause of death was respiratory failure with exudative DAD and massive capillary congestion, often accompanied by microthrombi despite anticoagulation. Ten cases showed superimposed bronchopneumonia. Further findings included pulmonary embolism ($n = 4$), alveolar hemorrhage ($n = 3$), and vasculitis ($n = 1$). Pathologies in other organ systems were predominantly attributable to shock; three patients showed signs of generalized and five of pulmonary thrombotic microangiopathy. Six patients were diagnosed with senile cardiac amyloidosis upon autopsy. Most patients suffered from one or more comorbidities (hypertension, obesity, cardiovascular diseases,
and diabetes mellitus). Additionally, there was an overall predominance of males and individuals with blood group A (81% and 65%, respectively) [428]. Myocardial hypertrophy was observed in 71% of COVID-19 cases, and liver pathologies such as liver steatosis were observed in 41% and liver shock necrosis in 29% of COVID-19 cases. The cytoplasm of kidney podocytes, endothelial cells, and proximal tubular epithelial cells contained multiple vesicles revealed by electron microscopy [428]. At higher magnification, the vesicles contain double membranes. Similar multiple vesicles were earlier observed in AD brain and in tryptamine-treated human neuronal cells by Paley, 2011 [50]. In summary, the findings provide an insight into the complexity of COVID-19 pathophysiology. SARS-CoV-2 substantially contributed to fatality in all cases, but the authors postulate a multifactorial cause of death, with COVID-19 as a contributory factor in multimorbid patients [428]. Major findings that imply an impaired microcirculation include pulmonary capillarostasis and the presence of microthrombi in the lungs and kidneys despite anticoagulation. The findings corroborate clinical and epidemiological data on cardiovascular morbidity and disease outcome and add amyloid transthyretin (ATTR) amyloidosis as a risk factor. Of note, ATTR is thyroxine-binding prealbumin, while serum thyroxine was lower in severe COVID-19 compared to non-severe cases and healthy individuals [372].

Barton et al. report the findings of two complete autopsies of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)-positive individuals who died in Oklahoma (United States) in March 2020 [419]. A 77-year-old obese man with a history of hypertension, splenectomy, and 6 days of fever and chills died while being transported for medical care. He tested positive for SARS-CoV-2 on postmortem nasopharyngeal and lung parenchymal swabs. Autopsy revealed DAD, chronic inflammation, and edema in the bronchial mucosa. A 42-year-old obese man with a history of myotonic dystrophy developed abdominal pain followed by fever, shortness of breath, and cough. Postmortem nasopharyngeal swab was positive for SARS-CoV-2; lung parenchymal swabs were negative. Autopsy showed acute bronchopneumonia with evidence of aspiration. Neither autopsy revealed viral inclusions, mucus plugging in airways, eosinophils, or myocarditis [419]. Bacterial cultures (aerobic/anaerobic) of the lung tissue grew nontoxigenic E. coli, Candida tropicalis, and Proteus mirabilis from the 42-year-old patient. In complete autopsy of the 77-year-old patient, there was hepatic centrilobular steatosis and remote cholecystectomy, and in the 42-year-old, hepatic cirrhosis and advanced remote cholecystectomy were revealed. Coronary artery disease was found in both patients.

Ackermann et al. reported in 2020 that the lungs from patients with COVID-19 showed distinctive vascular features, consisting of severe endothelial injury associated with the presence of intracellular virus and disrupted cell membranes. Histologic analysis of pulmonary vessels in patients with COVID-19 showed widespread thrombosis with microangiopathy. Alveolar capillary microthrombi were nine times as prevalent in patients with COVID-19 as in patients with influenza \( (P < .001) \). In lungs from patients
with COVID-19, the amount of new vessel growth, predominantly through a mecha-
nism of intussusceptive angiogenesis, was 2.7 times as high as that in the lungs from pa-
tients with influenza ($P < .001$) [429]. In this study, pulmonary autopsy specimens from
seven patients who died from respiratory failure caused by SARS-CoV-2 infection were
compared with lungs from seven patients who died from pneumonia caused by influenza
A virus subtype H1N1, a strain associated with the 1918 and 2009 influenza pandemics
[429].

Archer et al. described in 2020 the differences between COVID-19 pneumonia,
ARDS, and high-altitude pulmonary edema (HAPE) [430]. Although 80% of people
with COVID-19 have a minor, acute respiratory infection, the mortality range was
from 2% to 7%. Patients with COVID-19 pneumonia may decompensate because of
hypoxemic respiratory failure. Autopsy data show inflammation, DAD, alveolar fluid
accumulation, and occasional hyaline membranes, consistent with ARDS [430].
Recently, HAPE physiology was proposed to explain the edema and hypoxemia in
COVID-19 pneumonia.

Fried et al. reported four cases (positive SARS-CoV-2 testing) that illustrate a variety
of cardiovascular presentations of COVID-19 infection [431]. Case 1: A 64-year-old
woman is presented with COVID-19 pneumonia and differential diagnosis included
myopericarditis and cardiac amyloidosis. Case 2: A 38-year-old man with a history of
type 2 diabetes mellitus presented with 1 week of cough, pleuritic chest pain, and pro-
gressive shortness of breath to an outside hospital. The patient had a bradycardic arrest
lasting 6 min. Case 3: A 64-year-old woman with a nonischemic cardiomyopathy, atrial
fibrillation, hypertension, and diabetes mellitus presented with a nonproductive cough
and shortness of breath for 2 days. On arrival, she was afebrile, with blood pressure
153/120 mm Hg, heart rate 100 bpm, and oxygen saturation 88%. Case 4: A 51-year-
old man with a history of heart transplantation in 2007 and renal transplantation in
2010 presented with intermittent fever, dry cough, and shortness of breath for 9 days.
He denied any recent travel or sick contacts. His outpatient immunosuppression included
tacrolimus 5 mg twice daily, mycophenolate mofetil 250 mg twice daily, and prednisone
5 mg daily [431].

9.4 Conclusions

Metabolic profiling of sera from COVID-19 patients reveal altered metabolism of
choline, benzoic acid, hippurate, catechol, tryptophan, and shikimate metabolic pathway
compared to healthy subjects. Some of these metabolic alterations have a similar trend in
COVID-19 and non–COVID-19 patients. The data on hippurate serum content are
consistent with increased odds of having metabolic syndrome in COVID-19 patients.

Table 9.1 includes the data on positivity for the virus related to COVID-19 in
different zip codes (A zip code is a postal code used by the United States Postal Service)
mainly in Miami-Dade, Florida. Analysis of these data shows no direct corelation between the population density (density of population per square miles) and COVID-19 virus positivity.

Complete autopsy examinations may indicate that manifestations in different organs of COVID-19 patients are caused by chronic and acute toxicity. This suggestion is consistent with the data on serum metabolic profiling of COVID-19 patients. Some COVID-positive patients died with, but not of COVID-19.